IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Christoph KIRSCH, et al. Confirmation No.: 3234

Application No.: 09/831,272 Examiner: MARVICH, Maria

Date Filed: August 15, 2001 Group: 1633

For: CHIMERIC PROMOTERS CAPABLE OF MEDIATING GENE EXPRESSION IN PLANTS UPON PATHOGEN INFECTION AND USES THEREOF

37 C.F.R. § 1.132 DECLARATION

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

I, Imre Somssich, PhD, declare as follows:

1. I am one of the named inventors and am familiar with patent application No. 09/831,272 entitled " CHIMERIC PROMOTERS CAPABLE OF MEDIATING GENE EXPRESSION IN PLANTS UPON PATHOGEN INFECTION AND USES THEREOF" (hereafter the '361 application) and the subject matter described therein.

2. I hold a PhD degree in Biology and currently am working in plant molecular biology. I am presently a senior research group leader at the Max-Planck-Institute for Plant Breeding, Dept. of Plant-Microbe Interactions, Cologne, Germany.

3. I have authored or coauthored ~80~ scientific papers, and ~2~ issued patents (PCT/EP98/02194, PCT/EP1999/008710).

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4. I have reviewed the Office Action dated September 12, 2006. I have been asked by patent counsel to discuss the cited references in relation to the instant invention and point out the differences and why these do not teach, suggest, or disclose our invention.

Independent Claim 2 is copied below:

Claim 2. A chimeric promoter capable of mediating local gene expression in plants upon pathogen infection comprising

(i) two or more *cis*-acting elements sufficient to direct elicitor-specific expression of a nucleic acid sequence, wherein a cis-acting elicitor responsive element consists of a nucleotide sequence of SEQ ID NO: 11, and

(ii) a minimal promoter.

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5. The Examiner rejects claims 2, 8 and 39 as being anticipated by van de Locht et al. (EMBO J., 1990, vol. 9(9), pp. 2945-2950). Claims 2, 8 and 39 were rejected as being unpatentable over van de Locht et al. in view of Peare and Williams (Nucleic Acids Research, 1988, vol. 16(17), pp. 8467-8486) and Searle et al. (MCB, 1985, vol. 5(6), pp. 1480-1489), and further in view of Comai et al. (Plant Molecular Biology, 1990, vol. 15(3), pp. 373-381).

The Examiner asserts that "it would have been obvious to one skilled in the art to duplicate the isolated promoter fragment that is elicitor responsive as taught by van de Locht et al., as taught by Peare and Williams and Searle et al., and Comai et al. because van de Locht et al. teach that a

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fragment of the PR2 promoter is responsible for strong elicitor mediated gene activation and because Pears and Williams and Searle et al. teach that multiple elements are more effective than single elements and Comai et al. teach that it is within the ordinary skill of the art to generate chimeric vectors in which larger promoter elements are duplicated."

6. I will first discuss van de Locht et al. This reference teaches that a 5' regulatory region comprising the DNA sequence between -168 to -52 of the PR2 promoter directs elicitor-specific expression of the GUS reporter gene. As noted by patent examiner, pPR2-10 in figure 5 comprises SEQ ID NO:11, and this region is sufficient to direct elicitor-specific expression. However, constructs pPR2-11 and pPR2-12 in the same figure also comprise SEQ ID NO:11, but the elicitor-specific expression of these promoters is at best drastically reduced or even completely abolished (see standard deviations given in parentheses in figure 5). This already illustrates that the major elicitor-response elements contained in pPR2-10 are located between -168 and -108 and do not include SEQ ID NO:11.

Moreover, our invention is novel over van de Löcht et al. since the SEQ ID NO:11 derived from the PR2 promoter behaves in part unexpectedly when employed in synthetic promoters as was demonstrated by Rushton et al. (The Plant Cell 14, 749-762, 2002). SEQ ID NO:11 is a composite element containing in one part the elicitor-response region (-76 to -52) of the PR2 promoter and in addition the expression enhancing PR2 promoter sequence, GGAACC (-51 to -44).

Rushton et al. teach that the elicitor-response region (-76 to -52) of the PR2 promoter (termed Box D short in figure 9), which is also contained in the pPR2-10, -11, -12 constructs described in van

de Löcht, allow elicitor-specific expression of the GUS reporter gene when fused solely as a tetramer to a minimal TATA-box containing region. A tetramer of SEQ ID NO:11 (termed Box D) on the other hand allows not only elicitor-specific expression of the GUS reporter gene, but in addition, more than 13-fold higher overall GUS expression than a tetramer of Box D short (compare expression levels in Rushton et al., figure 9). This enhancement of expression is not observed for SEQ ID NO:11 in the context of its own PR2 promoter (figure 5, van de Löcht) since pPR2-11 and pPR2-12 both comprise SEQ ID NO:11 yet show at best very low expression levels. Thus, this drastic increase in GUS expression, as demonstrated by Rushton et al., is not merely a consequence of multimerization of the elicitor response region within SEQ ID NO:11 but also requires the additional sequences outside of the defined elicitor response region. Since this additional function is only observed in the synthetic promoter constructs but is not detectable in its native PR2 promoter context precise identification of SEQ ID NO:11 was not at all obvious and could not have been anticipated from the data revealed in van de Löcht et al.

7. Next, I will discuss Pears and Williams. Although Pears & Williams (1998) teach that multimers of a promoter element (actually only one copy versus two were tested!) can enhance expression, the patent examiner is incorrect in his claim that: "Pears and Williams teach that heterologous promoter sequences inserted into promoters can mediate sufficient gene expression" (see Claims Rejections page 5 and 6). In fact, as demonstrated by Pears & Williams in their cited publication, the tested promoter element (designated oligo L from the CP1 gene promoter) was incapable of mediating expression when placed in front of a heterologous gene

37 C.F.R. § 1.132 DECLARATION
In re: Application of: KIRSCH et al.
Application No.: 09/831,272
Confirmation No.: 3234
Examiner: MARVICH, Maria
Page - 5 -

(namely the actin1 S gene), Oligo L could only restore induced expression on the CP2 gene. CP2 is the sister gene of CP1 and both have originally evolved from a common ancestral gene via gene duplication. CP2 expression behavior is identical to CP1, and it contains the same oligo L-like elements in its promoter. Thus, the promoter sequence used cannot be regarded as heterologous in this respect. However, even in this case, oligo L derived from CP1 could only functionally replace the oligo L-like elements from CP2 when placed precisely at a similar position within the CP2 promoter. Pearx & Williams teach that their oligo L promoter element (also as a multimer) is inactive when placed at a different position even within the CP2 promoter.
Our invention is novel over Pearx & Williams (1998) since our described synthetic element clearly functions as a pathogen-responsive element when placed upstream of a heterologous gene. Furthermore, the exact positioning of our element, and multimers hereof, within a TATA-box containing promoter is not critical for allowing pathogen-induced expression.
Thus, from the information learned from Pearx & Williams, it would not have been obvious that our synthetic element behaved as it did.

8. The next reference, Searle et al. (1985) showed that two highly sequence-related elements (termed MREs for metal regulatory elements) conferred zinc-dependent expression when placed upstream of an otherwise non-responsive gene (TK gene). One copy of such a MRE was inefficient whereas multimers increased zinc-dependent expression.
Although Searle et al. could demonstrate in the case of the MREs that multimers are more effective than monomers, one cannot generalize this nor can one derive from their studies that this will be

(continued)

the case. In fact, Lozoya et al. (The Plant journal 1, 227-234, 1991) teach in figure 6 and table 1 that whereas the light responsive CHS promoter elements (termed 1 and 2) in the context of their own promoter enable light-specific expression of the GUS reporter gene, a dimer of element 1 placed *in* both orientations in front of a TATA box region cannot mediate light responsiveness. Similarly, Logemann et al (Proc. Natl. Acad. Sci. USA 92, 5905-5909, 1995) teach that tetramers of three different response elements of the PAL promoter do not enhance light- or elicitor-specific expression.

9. The last reference, Comai et al. teaches that by joining portions to two different promoters (from the 35S promoter and mas promoter) synergy and distinct properties may be obtained. Duplication of the 35S enhancer region (-430 to -90) resulted in 2-fold enhancement of expression levels.

I have difficulty in seeing how their results impinge on our claims. Although duplication of promoter regions can enhance expression, Comai et al. teach that the range of expression levels of such chimeric promoters was very broad and thus unpredictable. Furthermore Comai et al. clearly also state (page 379) that; "reiteration of the 35S upstream region (the '35S enhancer') did not have a major effect on expression ...". Furthermore, as also referred to in their paper, the group of Odell et al. (Plant Molecular Biology 10: 263-272, 1988) found that duplication of the 35S promoter (region -392 to -55) did not enhance expression. Moreover, Comai et al. do not teach that increased pathogen responsiveness can be achieved by duplication of promoter elements.

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37 C.F.R § 1.132 DECLARATION
In re: Application of: KIRSCH et al.
Application No.: 09/831,272
Confirmation No.: 3234
Examiner: MARVICH, Maria
Page - 7 -

10. I further state that all statements made herein are of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with my knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Dr. Imre Somssich, Ph. D.

March 2, 2007
Date